ABSTRACT
This paper reports microfluidic platforms that enable screening for solid forms (salts, polymorphs) of pharmaceutical parent compounds (PCs) via diffusive mixing or solvent evaporation, and subsequent on-chip characterization using Raman spectroscopy. Key features of the reported platforms include combinatorial mixing to screen different conditions (24–48), improved solvent compatibility, minimal solvent loss, and improved control over solvent evaporation. These platforms allow for screening of many more conditions with a limited amount of PC (~10 mg), thus facilitating solid form screening early in the drug development process. We validated the various platforms using model compounds such as ephedrine and tamoxifen.

KEYWORDS: Pharmaceuticals, microfluidics, salt formers, combinatorial screening, evaporation, Raman spectroscopy

INTRODUCTION
A large proportion of time and money expended in the drug development process is consumed in investigating thousands of PCs for their biopharmaceutical properties. Most of these compounds fail to perform due to unacceptable toxicity, limited stability, and poor physicochemical properties [1]. Knowledge of physicochemical properties early in the drug development process can assist in early selection of PCs with a higher propensity for success. Elucidation of these physicochemical properties requires identifying all the available solid forms (polymorphs, salts) of the PCs early in the development phase. A key challenge in salt screening is assessing a large number of salt formers (SF) in a variety of crystallization conditions with limited material. Several parameters influence crystallization of salts, including SF identity, PC-to-SF ratios, solvents employed, pH, and temperature. Therefore, extensive screens are traditionally conducted in several conditions to identify salts with suitable properties. Automated robotic systems have been developed to execute high-throughput screens, improving efficiency and reducing turnaround time [2]. However, these robotic tools typically require ~5 mg of material per condition, i.e., ~0.5 g for a full screen of 100 conditions at 10 mg/ml in 50 µl wells. Such quantities typically are not available in the early stages of drug development. Microfluidics has the potential to screen PCs at a much earlier stage using significantly smaller amounts of materials: ~0.5 mg instead of ~0.5 g for a full screen. Previously, microfluidic platforms for combinatorial applications have been reported, for example for protein-antibody binding assays [2]. Also, microfluidic platforms for crystallization screening have been reported for the identification of crystallization conditions of proteins via free interface diffusion (FID) [3] and via controlled solvent evaporation [4]. However, these microfluidic platforms are not compatible with (i) the wide range of solvents typically used in PC crystallization screens [5] and (ii) on-chip characterization of solid forms. Overcoming these two hurdles would drastically enhance the applicability of microfluidics in pharmaceutical screening.

Here, we present microfluidic platforms that are compatible with a range of solvents (i.e., ethanol, methanol, isopropyl alcohol, acetonitrile), allow for combinatorial mixing of PC and SF solutions, and enable on-chip analysis of the resulting solid forms by Raman spectroscopy [6]. We validated the platforms with the known pharmaceuticals, ephedrine and tamoxifen.

EXPERIMENTAL DESIGN
The microfluidic platforms are designed to mix PC and SF solutions in arrays of 24 (4 x 6) or 48 (4 x 12) sub-microliter wells (90 to 200 nL/well). Four PC concentrations in a single solvent are allowed to mix combinatorially with 6 or 12 SF solutions, resulting in the screening of 24 or 48 unique conditions, respectively. Alternatively, a single PC concentration in a solvent can be subjected to different solvent evaporation rates. The microfluidic platform comprises of different layers depending on the desired method of crystallization (FID or evaporation). Figure 1(a1) shows a schematic of the layered design of a 2 x 2 array of wells of the evaporation-based microfluidic crystallization-screening chip.

**FID-based microfluidic platform**: The FID-based microfluidic platform comprises of polydimethylsiloxane (PDMS) fluid and control layers, sandwiched between cyclic olefin copolymer (COC) top and bottom layers. The fluid layer contains chambers for the PC and SF solutions. The control layer has 4 sets of valves, labeled 1-4 in Figure 1(a2), for fluid routing and mixing. Figure 2(a) shows an optical micrograph of a 4 x 12 array chip for FID crystallization.

**Evaporation-based microfluidic platform**: Several modifications were made to the FID platform to enable evaporation-based screening. The bottom COC layer was spin-coated with a thin layer of PDMS for reversible bonding. A thiolene (NOA 81) layer incorporating the evaporation channels was added. Through-holes in the bottom COC layer connect the fluid chambers to the respective evaporation channels. After mixing, the mixed solutions are transferred to an additional chamber in the fluid layer using valve set 5, as shown in Figure 1(a2). From our previous work on evaporation-based crystallization [2], we know that the rate of solvent evaporation is a function of the dimensions of the evaporation channel as follows:
where $D$ is the solvent diffusivity, $P^*$ is the solvent vapor pressure, $P_T$ is the total pressure, $\rho$ is the solvent density, $A_c$ is the cross-sectional area, $L$ is the length, and $MW$ is the molecular weight of the solvent. We implemented evaporation channels with different $A_c$ and $L$ to screen a range of evaporation rates (complete evaporation of ~100 nL of solvent in 10–50 hours).

**Chip operation:** All valves are closed at rest and open by applying negative pressure. Figure 1(c) shows the filling, mixing, and mixture transfer processes in a 2 x 2 set of wells. The chip is filled horizontally with PC solutions by actuation of valve set 1 and 3, Figure 1(c1), and SF solutions are introduced vertically by actuation of valve set 2, Figure 1(c2). Solutions in adjacent compartments are mixed via actuation of valve set 3, Figure 1(c3), followed by transfer of the mixture to the evaporation chamber via actuation of valve set 5, aided by the application of positive pressure to valve set 4, Figure 1(c4). The transferred solutions then are allowed to evaporate through the evaporation channels in the thiolene layer.

**RESULTS AND DISCUSSION**

Incorporation of solvent compatible and impermeable materials such as COC and thiolene, and reduction in the thickness of PDMS layers to less than 150 µm drastically improved solvent compatibility, by minimizing solvent absorption over long-term evaporation experiments, and rendered the chips compatible with on-chip analysis via Raman spectroscopy.

**FID-based platform validation:** Ephedrine dissolved in methanol (5 M) was mixed with the following six salt formers (all acids) dissolved in methanol (10 M each): hydrochloric, sulfuric, methane sulfonic, ethane sulfonic, nitric, and phosphoric acids. Figure 2 shows the results of this ephedrine salt screen, while Figure 3(a) shows on-chip Raman analysis of the five salts formed as well as analysis of identical salt crystals obtained off-chip to verify the identity of the on-chip crystals.

**Evaporation-based platform validation:** Tamoxifen dissolved in ethanol (0.12 M) was mixed with the following six salt formers (all acids) dissolved in ethanol (0.25 M): benzoic, fumaric, methane sulfonic, citric, tartaric, and succinic acids. Figure 3(b) shows Raman analysis of salt crystals formed on-chip and off-chip to verify their identity, as before.

**CONCLUSION**

In summary, we reported the design, fabrication, and validation of microfluidic array chips for screening and analysis of pharmaceutical salts employing FID and evaporation-based crystallization. The platform precisely meters and combinatorially mixes PC and SF solutions in arrays of 24-48 wells, using significantly smaller quantities of PC compared to traditional methods. Reduction of the PDMS layer thickness and use of solvent impermeable materials minimized solvent loss and enabled on-chip Raman spectroscopy. Using ephedrine and tamoxifen, we demonstrated that this microfluidic chip can be employed to screen for and identify multiple crystalline salt forms of PCs using only a limited amount of material. Information regarding shape and size is obtained using optical microscopy while information regarding the identity of the salt is obtained with on-chip Raman spectroscopy. Efforts are underway to: (a) use these platforms to screen for cocrystals, antisolvents, and (b) test their compatibility with on-chip X-ray analysis.
Figure 2: (a) On-chip salt screening of ephedrine in a 48-well microfluidic chip via diffusional mixing (FID). Rows 1-4 are filled with ephedrine. Columns are filled with the following SF: hydrochloric, sulfuric, methane sulfonic, ethane sulfonic, nitric, and phosphoric acids, respectively. The insets show salt crystals of ephedrine hydrochloride (b1), bisulfate (b2), mesylate (b3), esylate (b4), and dihydrogen phosphate (b5).

Figure 3: Raman spectroscopy data (a) of the five ephedrine salts formed in a 48 well diffusional mixing chip (solid lines) and off-chip (dashed lines); and (b) of the six tamoxifen salts formed in a 24 well evaporation based microfluidic chip (solid lines) and off-chip (dashed lines).

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REFERENCES

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